STATE OF THE FIBRINOLYTIC SYSTEM OF THE BLOOD OF RABBITS WITH EXPERIMENTAL ATHEROSCLEROSIS

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Euglobulin fibrinolysis is delayed, the fibrinase and antifibrinolytic activity of the blood is increased, and the fibrinogen concentration rises in rabbits after prolonged administration of cholesterol. The content of antiplasmins is not significantly changed.

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Prolonged administration of an atherogenic diet to animals lowers the intensity of fibrinolysis [1, 4, 7, 11, 14, 16, 17]. Some workers associate this with an increase in the content of antiplasmins [10] or fibrinase [8, 15].

The object of this investigation was to study the fibrinolytic and antiplasmin properties of the blood in experimental atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 120 sexually mature rabbits of both sexes. The first group of rabbits (55) received cholesterol with the diet in a dose of 0.5 g/kg body weight and the animals were sacrificed after 30-45, 90-120, and 170-190 days. The second group of rabbits (10) received cholesterol in a dose of 0.2 g/kg body weight for 7 months, and the third group (10 animals) the same for 11 months. The remaining animals (45) were controls.

Euglobulin fibrinolysis was determined in oxalated plasma by the method of Copley and co-workers [13]. The total antifibrinolytic activity was measured from the degree of inhibition of lysis by the test serum of clots obtained from the euglobulin fraction of dogs' plasma [9]. In the control when 0.1 ml 0.025 M $CaCl_2$ solution and 0.1 ml dog euglobulin solution were added to 0.2 ml physiological saline, lysis of the clots usually took place within 35-50 min. In the experimental sample the physiological saline was replaced by rabbit serum diluted 1:30 with physiological saline. The result was given as an index calculated by dividing the time of lysis of the clots in the experimental sample by the corresponding time for the control.

Antiplasmin activity of the serum was judged from the change in size of the zone of lysis of heated bovine fibrin films by fibrinolysin solution (400 units/ml), previously mixed with an equal volume of the test serum diluted 1:30 with physiological saline. The fibrin films were made in petri dishes [12]. The calculation was carried out by the formula:

$$\text{Antiplasmin activity} = \frac{(\text{control} - \text{experiment}) \cdot 100\%}{\text{control}}$$

Fibrinase in the blood plasma was determined by the method of Sigg and Duckert as modified by V. P. Baluda and co-workers [3]. The fibrinogen concentration was measured colorimetrically by the principle of the biuret reaction [2], the cholesterol concentration was determined by M. A. Levchenko's method [6], and β-lipoproteins were estimated by a modified turbidimetric method [5].

EXPERIMENTAL RESULTS

No changes in the blood fibrinogen level were found during the first 30-45 days of the experiment in rabbits receiving cholesterol in a dose of 0.5 g/kg. Slowing of euglobulin fibrinolysis was found only after 90-120 days. The pooled results of blood investigations of rabbits kept on an atherogenic diet for more

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TABLE 1. Fibrinogen Concentration and Indices of Blood Fibrinolytic Activity of Rabbits with Experimental Atherosclerosis

Test index	Control		Experimental		P
	n	M ± m	n	M ± m	
Fibrinogen concentration (in mg %)	38	127.6 ± 4.2	57	139.8 ± 3.4	< 0.05
Euglobulin fibrinolysis (in h)	39	8.2 ± 1.2	59	17.4 ± 1.2	< 0.001
Total antifibrinolytic activity (index)	23	2.57 ± 0.23	26	3.80 ± 0.18	< 0.001
Antiplasmin activity (in percent)	12	34.8 ± 4.4	16	29.7 ± 5.0	> 0.4
Fibrinase activity (in sec)	19	49.0 ± 1.8	24	61.1 ± 3.1	< 0.001

than 3 months are given in Table 1. In these animals the fibrinogen content was slightly raised, while spontaneous fibrinolytic activity was lowered. A direct correlation of moderate degree ($r = +0.48$, $P < 0.01$) was found between the time of euglobulin fibrinolysis and the concentration of β-lipoproteins in the blood, but no correlation could be found between the degree of fibrinolysis and the blood cholesterol level ($r = +0.19$; $P > 0.1$).

The results of determination of the total antifibrinolytic activity of the serum showed an increase in rabbits receiving cholesterol with the diet for a long time. By the use of this method the possible effect of fibrinogen of the tested blood on fibrinolysis was excluded because the analysis was carried out with serum. Consequently, the slowing of euglobulin fibrinolysis mentioned above was due not to an increase in the quantity of substrate (fibrinogen) but to inhibition of the enzyme reaction (fibrinolysis).

It might be supposed that the antifibrinolytic activity of the blood increases in experimental atherosclerosis on account of an increase in the content of antiplasmins (antifibrinolysins). However, a special method by which the antiplasmin property of the serum could be investigated with elimination of effects of antiactivators and fibrinase showed that no significant changes took place in the content of antiplasmins during cholesterol feeding of the rabbits. Meanwhile a significant increase in fibrinstabilizing (fibrinase) ability of the plasma was found. This may perhaps be the explanation for the high antifibrinolytic activity and delayed euglobulin fibrinolysis of the blood of the experimental rabbits. However, the possibility is not ruled out that, besides this, in atherosclerosis the content of substances inhibiting the activation phase of fibrinolysis (antiactivators) is changed. No reliable indication of any such compounds has yet been obtained. The mechanism of the increase in fibrinase activity of the blood in experimental atherosclerosis likewise remains unexplained.

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